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RESEARCH PAPER

Application of Bio P60 and Bio T10 alone or in combination to control Fusarium wilt of Hydroponic Melon

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ABSTRACT

The research aimed to determine the effect of single and combined applications of Bio P60 and Bio T10 in suppressing stem base rot and its effect on the growth and production of hydroponic melon. This research was conducted at Flos Hydroponic Organic at Bansari Village, Bansari District, Temanggung Regency from February to June 2023. Randomized Block Design was used with 6 replicates. The treatments were control (propamocarb hydrochloride), Bio P60, Bio T10, and a combination of Bio P60 and Bio T10 (1:1, v/v). Variables observed were incubation period, disease intensity, infection rate, area under disease progress curve (AUDPC), control effectiveness, plant length, number of leaves, fresh weight, leaf color, first flowering date, first fruit formation, number of fruits per plant, fruit weight per plant, and phenolic compounds qualitatively. The results showed that the combined treatment of Bio P60 and Bio T10 had the best effect indicated by delaying the incubation period, reducing disease intensity, reducing infection rates, reducing AUDPC values, increasing the value of control effectiveness, increasing plant length, number of leaves, plant fresh weight, leaf color, time of first flower appearance, time of fruiting, and fruit weight respectively of 31.25, 41.19, 13.33, 65.31, 55.61, 17.25, 5.57, 36.44, 11.47, 8.55, 9.63, and 22.92 % compared to control. The application of Bio P60, Bio T10, and the combination could increase the phenolic compounds (tannins, saponins, and glycosides) qualitatively in melon leaves.

Key words: Bio P60, Bio T10, hydroponic melon, secondary metabolites, stem rot

INTRODUCTION

Melon (*Cucumis melo* L.) is one of the famous fruits that has a financial value and promising possibilities from an industrial viewpoint (Manchali et al., 2021). Globally, melon production is very high as market demand has been on the rise due to strong promotion in various regions of the world (Khalid et al., 2021). In 2022, melon production in Indonesia decreased by 8.09% compared to 2021 (BPS, 2022). One of the constraints in melon production is stem base rot caused by the soil-inhabiting fungus *Fusarium oxysporum* (Imazaki & Kadota, 2019). This fungus causes wilting in various crops, including cantaloupe, cucumber, wax gourd, muskmelon, watermelon, and other melon varieties (Mahdikhani, 2016). The disease is characterized by the appearance of pale-yellow lesions on the stem base, which expand and spread on the stem and root. The colonized stem leads to the breakdown of cortical tissues (Cohen et al., 2015). Stem base rot can reduce melon yield by more than 50% (Hao et al., 2020).

Currently, manipulation efforts are nevertheless focused on the use of synthetic pesticides. The indiscriminate use of fungicides causes many problems, due to their hazzards to humans, the environment, flowers and animals (Tang et al., 2021; Pathak et al., 2022). It also causes the development of fungicide -resistant strain of the pathogen (Yu et al., 2023). The hazardous impacts of the usage of synthetic pesticides can be decreased by utilising organic commercial products (Lahlali et al., 2022). On the other hand, organic pesticides are very friendly to the environment and do not produce residual effects entering the plant tissue (Zhao et al., 2018; Abd-Elgawad & Askary, 2020).

Regarding disease management in melons, various factors related to climate change and community demand have led to changes in the agricultural system. Recently, integrated and environmentally friendly production models have developed rapidly. This integrated method includes the use of biological

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control agents such as *Trichoderma* sp. to control plant diseases. Dual culture tests to evaluate the antagonistic ability of this fungus against *Fusarium* resulted in 93% disease control (González et al., 2020).

However, this protection is difficult to obtain even when *Trichoderma*-based formulations are applied preventively, allowing prior root colonization of the antagonist. Several authors have reported remarkable reductions in disease rates when *Trichoderma* isolates were applied to watermelon and melon plants artificially infected with *Fusarium* (González et al., 2020). Another important effect related to *Trichoderma* is the ability of the different species of the genus to promote and stimulate plant growth, which has been widely studied in different plant species, including cucurbits (Marín-Guirao et al., 2016). However, our results (data not shown) showed that the inoculation of the two protective *Trichoderma* strains increased the root biomass compared to the control plants, as previously shown for this genus in numerous plant hosts. This suggests another relevant feature of the genus from a biotechnological point of view, its potential use as microbial fertilizer (Zhang et al., 2018).

Therefore, innovation in plant disease control is expected, particularly via utilizing secondary metabolites of organic agents. Commercial secondary metabolites can be used as an alternative (Khan et al., 2020). Two of the antagonists that produce secondary metabolites are *Pseudomonas fluorescens* and *Trichoderma harzianum*. Secondary metabolites produced by *P. fluorescens* P60 and *T. harzianum* T10 have been formulated under the names of Bio P60 and Bio T10, respectively. Bio P60 can result in 70–80% disease suppression (Soesanto et al., 2019a; 2021). Bio P60 produces hormones, antibiotics, and some enzymes and is safe for human beings (Soesanto et al., 2011; Mishra & Arora, 2018). Meanwhile, Bio T10 produces anti-fungal compounds such as chitinase and glucanase enzymes that can degrade cell walls (Reino et al., 2008; Guo et al., 2022).

The use of secondary metabolites to control stem base rot disease in melons is very important to study. Therefore, this study aimed to determine the impact of single or mixed use of Bio P60 and Bio T10 in suppressing stem base rot disease and the growth and production of hydroponic melon plants.

MATERIALS AND METHODS

Research Site. The study took place at the Temanggung Organic Hydroponic Floss located in Bansari Village, Bansari District, Temanggung Regency. The site is situated at an altitude of approximately 1200 m above sea level with temperatures ranging from 20–30 ºC. The annual rainfall in this region is recorded at 2000 mm/year according to the Temanggung Regency Government in 2021. Additionally, the research was carried out on andosol soil type. The experiment was conducted from February to June 2023.

Preparation of Bio P60 and Bio T10. Bio P60 was prepared using *P. fluorescens* P60 isolates (Soesanto et al., 2019b) in King's B (Himedia, USA) liquid medium in 250 mL Erlenmeyer flasks (Pyrex). The flasks were incubated for 2 × 24 h at room temperature and shaken (Daiki Orbital Shaker, Republic of Korea) at 150 rpm. The liquid medium was made by boiling 400 g of snail meat with 1 L of water and adding 2 g of commercial shrimp paste until boiling. It was then filtered, and the broth was put into a sterile jerry can, covered, and refrigerated (Soesanto et al., 2010). An isolate of *P. fluorescens* P60 (concentration 10^9 cfu mL^{-1}) was added to the snail broth and shaken for 3 days at room temperature at 150 rpm (Soesanto et al., 2013), and its density was adjusted to 10^9 cfu mL^{-1}.

Bio T10 was prepared using *T. harzianum* T10. The broken corn medium was prepared in plastic bags with a thickness of 0.05 microns and sterilized in an autoclave at 121 ºC for 20 min. *T. harzianum* T10 was inoculated into the corn medium at about 5–10 g and incubated at room temperature (25–26 ºC) until the medium was full of mycelia. Next, 0.25 L of coconut water plus 10 g rice flour in 1 L of water was prepared, sterilized at 121 ºC for 30 min, then put into sterile jerry cans and refrigerated. *T. harzianum* T10 on the broken corn medium (50 mg *Trichoderma* for each 200 mL of water) was inoculated into the coconut water and rice flour medium and shaken at 150 rpm for seven days. The density was calculated using a hemocytometer and adjusted to 10^8 conidia mL^{-1} (Soesanto et al., 2020).

Preparation of Planting Media and Melon Seedlings. The planting media used was cocopeat, filled into 5 L polybags. Each polybag was labeled according to the treatment and replication in the study, and then arranged in a 140 cm × 60 cm area. Seeding was done by sowing melon seeds of Chamoe variety, produced in Korea, that had been soaked and then put into the seed tray with 128 holes (2.7 × 2.7 × 4 cm^3 each hole).

Planting and Maintenance. Melon seedlings were ready to transplant when they had 4–5 leaves or were

10–12 days old. Planting was done in the afternoon around 4 PM to 5 PM. Maintenance was performed by manually controlling weeds, pruning branches, maintaining the cleanliness of the greenhouse, and spraying with insecticides. Additionally, the plants supported with plastic ropes to ensure they grew straight. Drip irrigation was done regularly, 3–6 times a day.

Fusarium Inoculation. In order to control the fusarium wilt disease that always found at Organic Hydroponic Floss, we did not doing artificially inoculation of the patogen. The Fusarium infected naturally to the plant.

Application of Bio P60 and Bio T10. The application of Bio P60, Bio T10, and fungicides was done by watering and carried out routinely once a week according to the dose and treatment. Fungicide treatment as a control was done with propamocarb hydrochloride at a concentration of 0.5 mL L^{-1}, applied at 100 mL per plant. Bio P60 was applied at a concentration of 10 mL L^{-1}, 100 mL per plant; and Bio T10 was applied at 20 mL L^{-1}, 100 mL per plant. Furthermore, the combined application of Bio P60 and Bio T10 was carried out in a ratio of 1:1 (v/v). The treatments were arranged using a Randomized Block Design with 4 treatments and 6 replicates.

Observation Variables. The observation variables consisted of the incubation period, disease intensity, infection rate, area under the disease progress curve (AUDPC), plant length, number of leaves, fresh weight, leaf color, time to first flower appearance, first fruit formation, number of fruits per plant, fruit weight per plant, and qualitative phenol compound test. The incubation period was measured from planting time until the first symptoms appeared (days after planting = DAP). Disease intensity was calculated using the formula:

$$DI = \left[\frac{\Sigma(v \times n)}{Z \times N}\right] \times 100\%$$

DI = Disease intensity (%);
n = Number of plants observed showing a certain score;
v = Score for plants experiencing symptoms of senescence;
Z = Number of all plants observed;
N = Number of plants observed.

Scoring used in the study, according to Jaiswal & Tiwari (2022), included: 0 = plants with no symptoms; 1 = plants with 1–5% symptoms; 2 = plants with 6–25% symptoms; 3 = plants with 26–50% symptoms; 4 = plants with 51–75% symptoms; and 5 = plants with 76–100% symptoms. The disease infection rate was calculated weekly using the van der Plank formula (1963) as follows.

$$r = \frac{2.3}{t2 - t1}\left(\log\frac{Xt}{1 - Xt} - \log\frac{X0}{1 - X0}\right)$$

AUDPC was calculated using formula (Paraschivu et al., 2013) as followed.

$$AUDPC = \sum_{i=1}^{n} \frac{(x_i + xi_{+1})}{2}(t_{i=1} - t_i)$$

AUDPC = Area under the disease progression curve;
X_i = Specific disease occurrence at the i-th measurement;
t_i = Time of the i-th observation;
n = Total observations.

Control effectiveness values were calculated using the formula modified by Pigeot et al. (2003) as followed.

$$E = \left[\frac{IP_k - IP_p}{IP_k}\right] \times 100\%$$

E = Effectiveness Value;
IP_K = Disease intensity in the control;
IP_P = Disease intensity in the treatment.

Observations of growth components in plant length were measured using a roller meter. Leaf number observations were made by counting the number of fully developed (open) leaves. Plant fresh weight observations were made by weighing all parts of the plant that had been removed from the polybag. Leaf color observations were made using the leaf color chart (color scale range 2 to 5) (Figure 1).

Observations of yield components at the time of first flowering were recorded when the plant produced its first flower. First fruit formation was recorded when the plant produced its first fruit. The number of fruits per plant was calculated from the total number of fruits that appeared per plant. Fruit weight per plant was measured by weighing all the fruits of each harvest according to the treatment. Qualitative analysis of tannin compounds was carried out using the gelatin test, saponins by the foam test method, and glycoside compounds by the Killer-Kiliani test (Chairul, 2003).

Data Analysis. Data were analyzed by analysis of variance (ANOVA) at the 95% confidence level. If there was a significant effect, the Honest Significant Difference (HSD) test was also used at the 5% level of error. The observation of the phenol test was done descriptively.

RESULTS AND DISCUSSION

Effect of Treatments on Pathosystem Components. Stem base rot, caused by *F. oxysporum*, results in rot at the base of the stem, followed by wilting of the plant. Symptoms of the disease begin with the appearance of leaf spots, then the lower leaves of the plant turn yellow, causing the leaf tissue to die (necrosis symptoms) and then dry out (Figure 2). Further symptoms include wilting of the upper part of the plant, and in advanced cases, the plant collapses and dies. Seblani et al. (2023) suggested that leaf spot is one of the symptoms observed early on the leaves. Spots begin to appear from the edge of the leaf and then expand and coalesce, resulting in late blight. Stewart et al. (2015) added that symptoms of stem base rot include leaf spots and necrosis, wet spots on the stem, and affected fruit.

Based on the incubation period data, the results showed that the combined treatment of secondary metabolites was the most effective in delaying the incubation period, which was 30.33 DAP, representing a 31.25% delay compared to the control. It is suggested that the secondary metabolites of *P. fluorescens* P60 and *T. harzianum* T10 contained in Bio P60 and Bio T10 have synergistic properties and can inhibit the development of pathogens. This is consistent with Poveda & Eugui (2022), who stated that the synergy between *Trichoderma* and bacteria provides more benefits than either alone and is a promising alternative for disease or pest control in modern agriculture. The secondary metabolites of *P.*

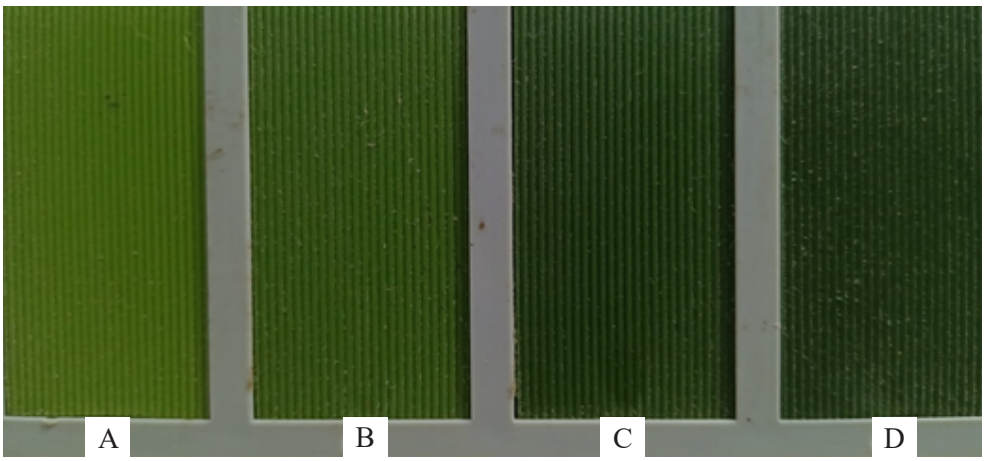

Figure 1. Leaf color chart with color scale range from A to D. A. Light green; B. Green; C. Dark green; D. Very dark green.

Figure 2. Symptoms of melon stem base rot, caused by *F. oxysporum*.

fluorescens contain bioactive compounds such as the antibiotic 2,4-diacetylphloroglucinol (Phl) and the enzyme chitinase (Ganeshan & Kumar, 2005; Mishra & Arora, 2018), while the bioactive compounds in the secondary metabolites of *T. harzianum* include the antibiotic peptaibol and the enzymes chitinase and glucanase (Khan et al., 2020; Guo et al., 2022). The secondary metabolites of *T. harzianum* T10 in the Bio T10 formula include β-(1-3) glucanase and chitinase enzymes. Khatri et al. (2017) and Loc et al. (2020) stated that in controlling pathogens, *T. harzianum* produces chitinase and β-1,3 glucanase enzymes, which can degrade chitin and glucan, components of the hyphal wall of several pathogenic fungi, resulting in cell wall lysis. Soesanto et al. (2022) proved that the application of secondary metabolites of *T. harzianum* T10 was able to reduce the intensity of Fusarium wilt in chili plants by 80.45%, while secondary metabolites of *P. fluorescens* P60 were able to reduce the incubation period by 75% in bacterial wilt disease of tomato plants (Soesanto et al., 2023).

The analysis of disease intensity showed a significant difference between the fungicide treatment and the application of Bio P60 and Bio T10 (Table 1). The best intensity suppression was achieved with the combined treatment of Bio P60 + Bio T10, which was able to suppress disease intensity by 41.19% compared to the control, aligning with the incubation period data. This is thought to be due to the combined use of secondary metabolites from biological agents that can act synergistically to inhibit the development of Fusarium wilt disease (Poveda & Eugui, 2022). Bio P60 contains secondary metabolites of *P. fluorescens* P60, including siderophores capable of binding Fe, resulting in Fe deficiency (Soesanto et al., 2010). Fe deficiency inhibits pathogen growth (Gu et al., 2020). Since Fe is required by pathogens as a nutrient source, the siderophores in the secondary metabolites of biological agents cause Fe to be unavailable to pathogens, thereby suppressing their growth. As a result, plants can grow optimally. The secondary metabolites of *T. harzianum* T10 in Bio T10 are postulated to inhibit pathogen growth because they produce antibiotics. Ordentlich et al. (1992) suggested that *T. harzianum* contains furanone group antibiotics that can inhibit the growth of spores and hyphae of pathogenic microbes. These antibiotics inhibit the growth of *F. oxysporum.* Soesanto et al. (2019a) proved that the application of Bio P60 and Bio T10 was able to reduce the intensity of stem base rot disease in bok choy (*Brassica rapa* var. *chinensis*).

The infection rate in both single and combined treatments of Bio P60 and Bio T10 was relatively slower compared to the control (Table 1), in accordance with the incubation period and disease intensity. The combined treatment of Bio P60 and Bio T10 was able to reduce the infection rate by 72.68% compared to the control. The lower infection rate of the single and combined treatments of Bio P60 and Bio T10 compared to the control indicated that all treatments were able to inhibit pathogen development. This was consistent with the disease intensity, which showed that Bio P60 and Bio T10 treatments, both single and combined, were able to reduce disease intensity compared to the control. This is probably because the secondary metabolites of *P. fluorescens* P60 and *T. harzianum* T10 contained in Bio P60 and Bio T10 can act as pathogen control agents. Soesanto et al. (2010) suggested that *P. fluorescens* P10 produces the antibiotic 2,4-diacetylphloroglucinol (Phl), which can inhibit wilt in potato and eggplant plants caused by *F. oxysporum.* The application of *T. harzianum* secondary metabolites can inhibit pathogen development and reduce disease intensity. This shows that *T. harzianum* produces compounds that can inhibit pathogen growth by degradation of cell wall. According to Khan et al. (2020) and Guo et al. (2022), *T. harzianum* produces β-(1-3) glucanase and chitinase enzymes in the control of plant pathogens, which can cause lysis of the cell walls of pathogenic fungi.

Table 1. Effect of treatments on pathosystem components of melon stem base rot

Treatments	Incubation period (Dai)	Disease intensity (%)	Infection rate (unit/days)	AUDPC (%-days)	Control effectivity (%)
Control	23.11 c	47.22 b	0.41	476.39 c	-
Bio P60	26.55 b	34.72 a	0.14	286.79 b	26.47
Bio T10	27.28 b	33.33 a	0.15	243.05 ab	29.41
Bio P60 + Bio T10	30.33 a	27.78 a	0.11	165.26 a	41.19

Data of disease intensity and infection rate were transformed by Arcsin√((x+0.5)). Numbers in the same column followed by different letters indicate significant differences according to the HSD test at 5% error level. Dai = days after inoculation.

Results of the experiment showed that the best AUDPC value occurred in the Bio P60 + Bio T10 combined treatment, which was reduced by 65.31% compared to the control, aligning with the incubation period, disease intensity, and infection rate data (Table 1). These results indicated that the Bio P60 + Bio T10 treatment had a synergistic effect against stem base rot disease. The higher the AUDPC value, the higher the disease intensity of the plant; conversely, the lower the AUDPC value, the lower the disease intensity. A fast incubation period causes a high rate of disease development, thus affecting the magnitude of the AUDPC value. This is supported by Soesanto et al. (2021), who stated that secondary metabolites can suppress pathogen development, thereby reducing infection rates. The high AUDPC value in the control treatment was due to the absence of secondary metabolites, which resulted in *F. oxysporum* being able to infect plants without inhibitors and develop well, causing stem base rot disease. The high or low AUDPC value illustrates the corresponding development of plant diseases.

Based on the calculation of disease control effectiveness values, the highest control effectiveness value was obtained in the combined treatment of secondary metabolites (Bio P60 + Bio T10) at 55.61% compared to the control (Table 1). This is in line with the low disease intensity and low AUDPC value. The higher the effectiveness value obtained, the better the control's impact in reducing the intensity of stem base rot disease. The combined treatment of secondary metabolites is the best stem base rot control, with the highest effectiveness value compared to other treatments. This is thought to be due to the presence of more than one compound that enhances resistance, causing increased plant resistance and inhibiting disease development (Table 3). Thambugala et al. (2020) stated that the combination of inter-microbial antagonists has three possibilities: synergistic, additive, and antagonistic. Liu et al. (2018) and Yu et al. (2022) added that the use of a combination of biological agents will suppress the spread of disease better than the use of biological agents alone.

Effect of Treatments on Growth Components. Based on the analysis, the length of melon plants was significantly different (Table 2). The combined treatment of Bio P60 + Bio T10 showed the best results, producing the longest plant compared to other treatments. The combined treatment of Bio P60 + Bio T10 increased plant length by 17.25% compared to the control (Table 2). The Bio P60 and combined Bio P60 + Bio T10 treatments were able to increase plant length, presumably because the secondary metabolites contain growth hormones. Bio P60 contains secondary metabolites from *P. fluorescens* P60 bacteria that are PGPR (Plant Growth Promoting Rhizobacteria). This is in line with Soesanto et al. (2010), which states that *P. fluorescens* P60 is capable of being PGPR, thus stimulating root growth and inhibiting fungi and bacteria. Orozco-Mosqueda et al. (2023) added that *P. fluorescens* produces growth hormones, including auxin, gibberellin, and cytokinin. Bio T10 contains secondary metabolites of *T. harzianum* T10 that are PGPF (Plant Growth Promoting Fungi). This is in accordance with Kumar et al. (2022), who state that *T. harzianum* T10 plays a role in promoting plant growth and development because it has a PGPF mechanism. Ganeshan & Kumar (2005) suggested that *T. harzianum*, apart from being an antagonist, also acts as a PGPF that produces growth hormone.

The number of melon plants leaves showed significantly different results. The combined treatment of Bio P60 and Bio T10 increased the number of leaves by 5.57% compared to the control. This is probably because the secondary metabolites of *P. fluorescens* P60 and *T. harzianum* T10 contain growth hormones, thus increasing plant growth. Elekhtyar (2015) stated that *P. fluorescens* can act as a biological agent and PGPR, stimulating plant growth and inhibit fungal growth. Secondary metabolites of *T. harzianum* play a role in increasing plant growth and development by stimulating plant fertilization. This occurs due to interactions with plants that spur growth hormones (Kalay et al., 2019; Ismi et al., 2022).

The analysis in Table 2 revealed that the Bio P60 and Bio T10 treatments had a significant effect on plant fresh weght compared to the control. The highest plant fresh weight was observed in the combined treatment of Bio P60 + Bio T10, which was 32.83% higher than the control. This was thought to be due to secondary metabolites produced by *P. fluorescens* P60 and *T. harzianum* T10, which contain growth hormones that increase plant growth and yield as well as inhibit the growth of pathogens. This is in accordance with Elekhtyar (2015), who stated that *T. harzianum* can accelerate plant growth by stimulating plants and producing gibberellins, IAA, and benzylaminopurine, thus affecting plant resistance. Edelmann (2022) added that IAA can increase the growth of lateral roots, multiply shoots, and increase the biomass of plant shoots. Ahemad & Kibret (2014) suggested that *P. fluorescens* is a phytohormone-producing microbe, especially IAA, and can increase plant growth by

regulating hormone balance in infected plants. This allows the combination of secondary metabolites to increase plant fresh weight.

Data in Table 2 showed that the colour of the leaves on melon plants had significantly different results. The Bio P60 + Bio T10 treatment resulted in a more intense leaf colour, with a value of 3.78, which is 11.54% higher compared to the control. The higher level of chlorophyll available in the leaves results in a more intense green pigment and increased photosynthesis. Sufficient chlorophyll content can enhance plant growth. This is attributed to the secondary metabolites of *T. harzianum* and *P. fluorescens,* which contain growth hormones (Soesanto et al., 2011b; Kumar et al., 2022). Silva et al. (2023) suggested that *T. harzianum* can decompose organic matter, such as N, P, and K, ensuring these nutrients are sufficient and support plant growth. Bean et al. (2022) added that *Trichoderma* sp. can increase the number and width of leaves and leaf chlorophyll levels since *Trichoderma* sp. synthesizes cytokinin hormones.

Effect of Treatments on Yield Components. Based on Table 3, the first flowering date showed significantly different results. The time required for the plants to flower for the first time was faster in the plants with the combined treatment of Bio P60 and Bio T10 compared to the control. The time to first flower appearance in the Bio P60 + Bio T10 treatment was 8.55% faster than the control. The application of Bio P60 and Bio T10, both single and in combination, can accelerate the flowering time of melon plants, allegedly because they contain growth hormones. This is in accordance with Lakhdari et al. (2022), who stated that the secondary metabolites of *T. harzianum* contain gibberellin and auxin, which play a role in root and stem elongation, stimulate flowering and fruit growth, and increase plant growth. Furthermore, Tsukanova et al. (2017) mentioned that *P. fluorescens* produces secondary metabolites that contain hormones that can increase plant growth, including auxin, gibberellin, and cytokinin. The time required for plants to flower for the first time was the longest in the control treatment with fungicide.

Table 3 showed that the time of first fruit emergence was significantly different. Melon plants with the Bio P60 + Bio T10 combined treatment bore fruit 9.63% faster compared to the control, in line with the appearance of the first flower. This is thought to be due to the provision of secondary metabolites that can spur growth, thus accelerating the emergence of fruit. Secondary metabolites of *P. fluorescens* and *T. harzianum* from biological agents produce hormones that can accelerate fertilization. This is supported by Tsukanova et al. (2017), who stated that the secondary metabolites of *P. fluorescens* contain hormones that can accelerate growth, including the age of plants when flowers, fruit, and harvest appear. Secondary metabolites of *T. harzianum* produce hormones that can spur growth. Lakhdari et al. (2022) and Silva et al. (2023) mentioned that *T. harzianum* can increase

Table 2. Effect of treatments on melon growth component

Treatments	Plant length (cm)	Number of leaves	Fresh weight per plant (g)	Leaf color scale
Control	86.59 b	16.94 b	553.33 b	3.38 b
Bio P60	99.92 ab	17.66 ab	675.00 ab	3.66 ab
Bio T10	90.49 ab	17.16 ab	543.33 ab	3.55 ab
Bio P60 + Bio T10	101.53 a	17.88 a	735.00 a	3.77 a

Numbers in the same column followed by different letters indicate significant differences according to the HSD test at 5% error level

Table 3. Effect of treatments on melon yield components

Treatments	First flower (DAP)	First fruit (DAP)	Number of fruits	Fruit weight (g)
Control	23.39 c	46.72 c	5.83 a	453.92 b
Bio P60	22.55 b	44.44 b	6.17 a	525.75 ab
Bio T10	21.83 a	43.83 ab	6.50 a	511.41 ab
Bio P60 + Bio T10	21.39 a	42.22 a	6.83 a	557.94 a

Numbers in the same column followed by different letters show significant differences at the BNJ 5% error level. DAP = days after planting.

plant growth and the absorption of minerals and nutrients from the soil. Abdullah et al. (2021) added that *T. harzianum* plays a role in spurring plant growth and development, such as stimulating fertilization. This is due to interactions with plants that spur growth hormones. This allows the combination of secondary metabolites to accelerate fertilization. However, the application of Bio P60 and Bio T10 has not significantly affected the number of fruits per plant (Table 3). Despite this, Bio P60 and Bio T10 treatments, both single and combined, produce more fruit than the control. This condition is in line with the flowering data. This is probably because the administration of secondary metabolites is not balanced with proper artificial pollination techniques. In melon cultivation, fertilization of melon plants requires pollination. In pollination, it is necessary to pay attention to the time and technique of pollination. According to Mondo et al. (2022), the optimum time for pollination is when the flower is in an optimal state, which is when the flower blooms. The highest crossing success in melon fruit is achieved between 06.00-07.00 a.m. because of the high level of humidity in the morning (Wang et al., 2017). According to Lord & Russell (2002), pollination is done by placing pollen from male flowers onto the stigma of female flowers. Placement should be done carefully to avoid damage and injury to the stigma and prevent infection with diseases.

The fruit weight data showed significant differences as well. The highest fruit weight was in the combined treatment of Bio P60 + Bio T10, which increased by 22.92% compared to the control, aligning with other data (Table 3). This is probably because Bio P60 and Bio T10 contain secondary metabolites of *P. fluorescens* P60 and *T. harzianum* T10, which can increase fruit weight. *P. fluorescens* P60, as a PGPR, supplies nutrients and growth hormones, stimulating root elongation, which results in increased root wet and dry weight, leading to better plant growth and higher yields (Tsukanova et al., 2017). PGPR functions as a nutrient supplier, antibiosis agent, and growth hormone producer. The combination of these functions can stimulate root elongation. Longer roots result in better plant growth, thereby increasing plant yields (Silva et al., 2023). Soesanto et al. (2022) mentioned that *T. harzianum* T10 can stimulate plants to produce gibberellins and auxins, which play a role in root and stem elongation, as well as in flowering and fruit growth. *T. harzianum* can produce antifungal compounds that penetrate the host plant and form a barrier to the entry of soil-borne pathogenic fungi, thereby inhibiting pathogenic fungi. With the inhibition of pathogenic fungi, nutrient and water transport becomes efficient, resulting in improved plant growth and higher crop yields (Abdullah et al., 2021; Silva et al., 2023).

Qualitative Analysis of Phenol Content. The results of the qualitative analysis of phenol content showed varying levels of tannin, saponin, and glycoside compounds across all treatments (Table 4). In the tannin test, the colour change is indicated by dark blue or blue-black (Chairul, 2003). The best tannin results were observed in the Bio P60, Bio T10, and Bio P60 + Bio T10 treatments compared to the control. The saponin test was indicated by foam formation, and the application of secondary metabolites from *P. fluorescens* P60 and *T. harzianum* T10 increased the foam height. The glycoside test was indicated by color changes to brownish red, blue, or mauve (Chairul, 2003). The best glycoside results were consistent with the tannin and saponin tests, showing increases due to the application of secondary metabolites from *P. fluorescens* P60 and *T. harzianum* T10. It is suspected that the application of secondary metabolites from biological agents can increase the content of tannins, saponins, and glycosides in plant tissues. Abiotic and biotic factors can increase plant secondary metabolites, including tannins (Thakur et al., 2019).

The tissue analysis results revealed that the application of Bio P60, Bio T10, and their combination had the highest amount of phenol compounds compared to the control (Table 4). Phenol compounds are component of plant biochemical resistance to plant pathogen attack (Zhang et al., 2022). This indicates that Bio P60 and Bio T10, containing secondary metabolites, support biochemical plant resistance to

Table 4. Effect of treatments on qualitative phenol content analysis in melon plants

Tretaments	Tannins	Saponin	Glycoside
Control	+	+	++
Bio P60	++	+++	+++
Bio T10	+++	++	+++
Bio P60 + Bio T10	+++	++	++

+ = Little; ++ = Quite a lot; +++ = A lot.

Fusarium pathogen infection. This aligns with Jacoby et al. (2021), who stated that plants produce a number of chemically diverse secondary metabolites, which exert a bioactive influence on microbes.Plant secondary metabolites affect the composition and function of the microbiome. Chen et al. (2023) also suggested that plant resistance is indicated by the formation of chemical compounds that can prevent the growth and development of pathogens. These compounds, called secondary metabolites, include alkaloids, phenols, flavonoids, glucose, and phytoalexins. These compounds are produced by plants in response to pathogen attack or an unfavorable environment and serve as the plant's natural defence against pathogens. The combined application of Bio P60 and Bio T10 showed phenol compounds not significantly different from the single application of Bio P60 and Bio T10 but higher than the control. The difference in phenol compounds is thought to be due to the varying ability of each plant to produce phenol compounds. The production of secondary metabolites differs depending on each strain of bacteria according to the ecological conditions of the bacteria (Jacoby et al., 2021).

CONCLUSION

The combination of Bio P60 and Bio T10 was the best treatments for delaying the incubation period by 31.25%, suppressing disease intensity, infection rate, and AUDPC value by 41.19%, 13.33%, and 65.31%, respectively, and increasing the control effectiveness value by 55.61% compared to the control. The combined Bio P60 and Bio T10 treatment was also the best at increasing plant length, leaf number, plant fresh weight, leaf color, time to first flower appearance, time to fruiting, and fruit weight by 17.25%, 5.57%, 36.44%, 11.47%, 8.55%, 9.63%, and 22.92%, respectively compared to the control. The combined application of Bio P60 and Bio T10 qualitatively increase the phenol compound content compared to the control.

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AUTHORS' CONTRIBUTIONS

LS was the conceptualizer of the study and the first author of the paper. IVN was the data collector, while MWR performed the statistical analysis. EM performed the laboratory analysis. NWAL was responsible for the greenhouse facilities. RFR was the data interpreter and finalized the paper writing and author for correspondence as well.

COMPETING INTEREST

The authors declare that they have no known competing financial interest or personal relationship that could have appeared to influence the work reported in this paper.

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